```
=> d his

     (FILE 'HOME' ENTERED AT 13:56:25 ON 23 JAN 2000)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, USPATFULL, WPIDS' ENTERED AT
     13:56:46 ON 23 JAN 2000
L1          1383 S SHI Q?/AU
L2         15445 S LIU S?/AU
L3           592 S LING M?/AU
L4             4 S L1 AND L2 AND L3
L5             1 DUP REM L4 (3 DUPLICATES REMOVED)
L6             8 S 98824
L7             0 S L6(3A)ATCC
L8             0 S ATCC(W)L6
L9         19076 S TROPONIN
L10          704 S CTNI
L11         5737 S L9(W)I
L12          125 S FRAGMENT#(5A)(L10 OR L11)
L13            6 S N(W)TERMINAL(5A)L12
L14            0 S N(W)TERMINUS(5A)L12
L15            6 S L13 NOT L4
L16            5 DUP REM L15 (1 DUPLICATE REMOVED)
L17        62692 S EXPRESSION VECTOR#
L18           42 S L17 AND (L10 OR L11)
L19            7 S L18 AND N(W)TERMINAL
L20            7 S L19 NOT L4
L21            6 S L20 NOT L15
L22            6 DUP REM L21 (0 DUPLICATES REMOVED)
L23           40 S L1-3 AND L9
L24           36 S L23 NOT L4
L25           36 S L24 NOT L13
L26           36 S L25 NOT L22
L27           20 DUP REM L26 (16 DUPLICATES REMOVED)
```

1 cite (authors)

5 cites

6 cites

20 cites

=> d bib abs 15

L5 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
AN 1999317100 MEDLINE
DN 99317100
TI Degradation of cardiac troponin I in serum complicates comparisons of cardiac troponin I assays.
AU **Shi Q**; **Ling M**; Zhang X; Zhang M; Kadijevic L; **Liu S**; Laurino J P
CS Spectral Diagnostics Inc., 135-2 The West Mall, Toronto, Ontario, Canada M9C 1C2.. qshi@ica.net
SO CLINICAL CHEMISTRY, (1999 Jul) 45 (7) 1018-25.
Journal code: DBZ. ISSN: 0009-9147.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199909
EW 19990904
AB BACKGROUND: Up to a 20-fold variation in serum cardiac troponin I (cTnI) concentration may be observed for a given patient sample with different analytical methods. Because more limited variation is seen for control materials and for purified cTnI, we explored the possibility that cTnI was present in altered forms in serum. METHODS: We used four recombinantly engineered cTnI fragments to study the regions of cTnI recognized by the Stratus(R), Opus(R), and ACCESS(R) immunoassays. The stability of these regions in serum was analyzed with Western blot. RESULTS: The measurement of several control materials and different forms of purified cTnI using selected commercial assays demonstrated five- to ninefold variation. Both the Stratus and Opus assays recognized the N-terminal portion (NTP) of cTnI, whereas the ACCESS assay recognized the C-terminal portion (CTP) of cTnI. Incubation of recombinant cTnI in normal human serum produced a marked decrease in cTnI concentration as determined with the ACCESS, but not the Stratus, immunoassay. Western blot analysis of the same samples using cTnI NTP- and CTP-specific antibodies demonstrated preferential degradation of the CTP of cTnI. CONCLUSIONS: The availability of serum cTnI epitopes is markedly affected by the extent of ligand degradation. The N-terminal half of the cTnI molecule was found to be the most stable region in human serum. Differential degradation of cTnI is a key factor in assay-to-assay variation.

=> d bib abs l16

L16 ANSWER 1 OF 5 USPATFULL
AN 1999:104806 USPATFULL
TI Diagnostic for determining the time of a heart attack
IN Buechler, Kenneth Francis, San Diego, CA, United States
McPherson, Paul H., Encinitas, CA, United States
PA Biosite Diagnostics Incorporated, San Diego, CA, United States (U.S. corporation)
PI US 5947124 19990907
AI US 1997-821888 19970321 (8)
PRAI US 1997-39545 19970311 (60)
DT Utility
EXNAM Primary Examiner: Yu, Mickey; Assistant Examiner: O'Hara, Kelly
LREP Lyon & Lyon LLP
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 3084
AB The present invention relates to methods for determining the time of a myocardial infarction in a patient by measuring the ratio of oxidized to reduced troponin I in a blood sample obtained from the patient. This ratio is measured through the use of two or more distinct components which specifically bind oxidized troponin I, reduced troponin I, and/or both forms of troponin I present in the blood sample. Each distinct component may be an antibody or an antibody fragment. The measured ratio reflects the time elapsed from the time of the myocardial infarction.

=> d kwic

L16 ANSWER 1 OF 5 USPATFULL

=> d bib abs l16 2

L16 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 1999:372198 CAPLUS
DN 131:55266
TI The troponin C-troponin I hydrophobic interactions in the formation of functional troponin and in the Ca2+ regulation of muscle contraction
AU Vassylyev, Dmitry G.; Takeda, Soichi; Maeda, Yuichiro
CS Cent. Res. Lab., Matsushita Electr. Ind. Co., Ltd., Japan
SO Seibutsu Butsuri (1999), 39(3), 144-147
CODEN: SEBUAL; ISSN: 0582-4052
PB Nippon Seibutsu Butsuri Gakkai
DT Journal; General Review
LA Japanese
AB A review with 22 refs. At. structure of troponin C (TnC) in complex with **N-terminal fragment** of **troponin** **I** (TnI1-47) detd. at 2.3 .ANG. resoln. revealed the compact globular conformation of the TnC mol., which is likely to exist within the intact troponin (Tn). The TnI1-47 long .alpha.-helix joins two domains of TnC by polar interaction, while its amphiphilic portion is tightly bound in the hydrophobic cleft of the C-domain of TnC through 38 van der Waals interactions. The model was proposed for another TnI amphiphilic .alpha.-helical segment, which binding/release to/from the regulatory N-domain of TnC would actually regulate the acto-myosin ATPase.

=> d bib abs l16 3

L16 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 1998:288983 CAPLUS
DN 129:51070
TI Crystal structure of troponin C in complex with troponin I fragment at 2.3-.ANG. resolution
AU Vassylyev, Dmitry G.; Takeda, Soichi; Wakatsuki, Soichi; Maeda, Kayo; Maeda, Yuichiro
CS Central Research Laboratories, International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd., Kyoto, 619-02, Japan
SO Proc. Natl. Acad. Sci. U. S. A. (1998), 95(9), 4847-4852
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB Troponin (Tn), a complex of 3 subunits (TnC, TnI, and TnT), plays a key role in Ca2+-dependent regulation of muscle contraction. To elucidate the interactions between the Tn subunits and the conformation of TnC in the Tn complex, the authors detd. the crystal structure of TnC (2-Ca2+ bound state) in complex with the N-terminal fragment of TnI (TnI1-47). The structure was solved by the single isomorphous replacement method in combination with multiple wavelength anomalous dispersion data. The refinement converged to a crystallog. R factor of 22.2% (Rfree = 32.6%). The central, connecting .alpha.-helix obsd. in the structure of uncomplexed TnC (TnCfree) was unwound at the center (residues Ala-87, Lys-88, Gly-89, Lys-90, and Ser-91) and bent by 90.degree.. As a result, TnC in the complex had a compact globular shape with direct interactions between the N- and C-terminal lobes, in contrast to the elongated dumb-bell shaped mol. of uncomplexed TnC. The 31-residue long TnI1-47 .alpha.-helix stretched on the surface of TnC and stabilized its compact conformation by multiple contacts with both TnC lobes. The amphiphilic C-end of the TnI1-47 .alpha.-helix was bound in the hydrophobic pocket of the TnC C-lobe through 38 van der Waals interactions. The results indicated the major difference between Ca2+ receptors integrated with the other proteins (TnC in Tn) and isolated in the cytosol (calmodulin). The TnC/TnI1-47 structure implies a mechanism of how Tn regulates the muscle contraction and suggests a unique .alpha.-helical regulatory TnI segment, which binds to the N-lobe of TnC in its Ca2+ bound conformation.

=> d bib abs l16 4

L16 ANSWER 4 OF 5 MEDLINE DUPLICATE 1
AN 1999106500 MEDLINE
DN 99106500
TI The crystal structure of troponin C in complex with **N-terminal fragment** of **troponin I**. The mechanism of how the inhibitory action of troponin I is released by Ca(2+)-binding to troponin C.
AU Vassylyev D G; Takeda S; Wakatsuki S; Maeda K; Maeda Y
CS International Institute for Advanced Research, Central Research Laboratories, Matsushita Electric Industrial Co., Ltd., Kyoto, Japan.
SO ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1998) 453 157-67.
Journal code: 2LU. ISSN: 0065-2598.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
EW 19990402
AB Troponin (Tn), the complex of three subunits (TnC, TnI, and TnT), plays a key role in Ca2+ dependent regulation of muscle contraction. To elucidate the interactions between the Tn subunits and the conformation of TnC in the Tn complex, we have determined the crystal structure of TnC in complex with the N-terminal fragment of TnI (TnI1-47). The structure was solved by single isomorphous replacement method in combination with multiple wavelength anomalous dispersion data. The refinement converged to a crystallographic R-factor of 22.2% (R-free = 32.6%). The central, connecting alpha-helix observed in the structure of uncomplexed TnC (TnCfree) is unwound at the center and bent by 90 degrees. As a result, the TnC in the complex has a compact globular shape with direct interactions between the N- and C-lobes, in contrast to the elongated dumb-bell shaped molecule of uncomplexed TnC. The 31-residue long TnI1-47 alpha-helix stretches on the surface of TnC and stabilizes its compact conformation by multiple contacts with both TnC lobes. The amphiphilic C-terminal end of the TnI1-47 alpha-helix is tightly bound in the hydrophobic pocket of the TnC C-lobe through 38 van der Waals interactions. The results indicate the major difference between integrated (TnC) and isolated (calmodulin) Ca2+ receptors. The TnC/TnI1-47 structure suggests the model for a novel regulatory TnI segment bound to TnC and implies the mechanism of how Tn regulates the muscle contraction.

=> d bib abs l16 5

L16 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 1992:547767 CAPLUS
DN 117:147767
TI Biologically important interactions between synthetic peptides of the N-terminal region of troponin I and troponin C
AU Ngai, Sai Ming; Hodges, Robert S.
CS Dep. Biochem., Univ. Alberta, Edmonton, AB, T6G 2H7, Can.
SO J. Biol. Chem. (1992), 267(22), 15715-20
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The interaction between troponin I (TnI) and troponin C (TnC) plays a crit. role in the regulation of muscle contraction. In this study the interaction between troponin C and the N-terminal region of TnI was investigated by the synthesis of three TnI peptides (residues 1-40/Rp, 10-40, and 20-40). The regulatory peptide (Rp) on binding to TnC prevents the ability of TnC to release the inhibition of the acto-S1-tropomyosin ATPase activity caused by TnI or the TnI inhibitory peptide (Ip), residues 104-115. A stable complex between TnC and Rp in the presence of Ca2+ was demonstrated by polyacrylamide gel electrophoresis in the presence of 6M urea. Rp was able to displace TnI from a preformed TnI.cntdot.TnC complex. In the absence of Ca2+, Rp was unable to maintain a complex with TnC in benign conditions of polyacrylamide gel electrophoresis which demonstrates the Ca2+-dependent nature of this interaction. Size-exclusion chromatog. demonstrated that the TnC.cntdot.Rp complex consisted of a 1:1 complex. The results of these studies have shown that the N-terminal region of TnI (1-40) plays a crit. role in modulating the Ca2+-sensitive release of TnI inhibition by TnC.

=> d bib abs l22

L22 ANSWER 1 OF 6 USPATFULL
AN 1999:75500 USPATFULL
TI Methods and compositions for the use of apurinic/apyrimidinic endonucleases
IN Kelley, Mark R., Zionsville, IN, United States
Duguid, John, Brownsburg, IN, United States
Eble, John, Indianapolis, IN, United States
PA Advanced Research & Technology Institute, Bloomington, IN, United States (U.S. corporation)
PI US 5919643 19990706
AI US 1997-872719 19970611 (8)
PRAI US 1996-19561 19960611 (60)
US 1996-19602 19960611 (60)
DT Utility
EXNAM Primary Examiner: Patterson, Jr., Charles L.
LREP Arnold, White & Durkee
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 57 Drawing Figure(s); 21 Drawing Page(s)
LN.CNT 4677
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods and compositions for identifying, monitoring and treating premalignant and malignant conditions in a human subject. The present invention further discloses methods and compositions for determining cells undergoing apoptosis, and for increasing the efficacy of a cancer therapy. The methods involve the use of apurinic/apyrimidinic endonuclease (APE), independently, as a marker for (pre)malignant conditions and for apoptosis. Also described are polyclonal antibody preparations for use in methods for detecting APE and methods for modulating expression susceptibility of cells to apoptosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l22 1 kwic

L22 ANSWER 1 OF 6 USPATFULL
DETD . . . region (discussed below). Alternatively, treatment of the APE molecule with proteolytic enzymes, known as protease, can produces a variety of **N-terminal**, C-terminal and internal fragments. Examples of fragments may include contiguous residues of the APE sequence given in SEQ ID NO:2,. . .
DETD . . . DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an **expression vector**, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.
DETD . . . diagnostic or therapeutic purposes related independently to (pre)malignant and apoptotic states of a cell. Thus, the present invention also encompasses **expression vectors** designed to provide for the production of APE. In other aspects, it may be advantageous to decrease the production of. . .
DETD Similarly, any reference to a nucleic acid should be read as encompassing an **expression vector** and host cell containing that nucleic acid. In addition to diagnostic considerations, cells expressing nucleic acids of the present invention. . .
DETD . . . Gene
.alpha.-Fetoprotein
.tau.-Globin
.beta.-Globin
e-fos
c-HA-ras
Insulin
Neural Cell Adhesion Molecule (NCAM)
.alpha.1-Antitrypsin

H2B (TH2B) Histone
Mouse or Type I Collagen
Glucose-Regulated Proteins (GRP94 and GRP78)
Rat Growth Hormone
Human Serum Amyloid A (SAA)
Troponin I (TN I)
Platelet-Derived Growth Factor
Duchenne Muscular Dystrophy
SV40
Polyoma
Retroviruses
Papilloma Virus
Hepatitis B Virus
Human Immunodeficiency Virus
Cytomegalovirus
Gibbon APE Leukemia Virus

DETD (iv) Delivery of **Expression Vectors**

DETD There are a number of ways in which **expression vectors** may introduced into cells. In certain embodiments of the invention, the expression construct comprises a virus or engineered construct derived. . .

DETD One of the preferred methods for in vivo delivery involves the use of an adenovirus **expression vector**. "Adenovirus **expression vector**" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to. . .

DETD The **expression vector** comprises a genetically engineered form of adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kB, linear, double-stranded DNA. . .

DETD . . . under the appropriate conditions. The gene for virtually any polypeptide may be employed in this manner. The generation of recombinant **expression vectors**, and the elements included therein, are discussed above. Alternatively, the protein to be produced may be an endogenous protein normally. . .

DETD . . . placed on the use of antisense constructs, which require specific levels of identity to achieve hybridization. The lengthy discussion of **expression vectors** and the genetic elements employed therein is incorporated into this section by reference. Particularly preferred **expression vectors** are viral vectors such as adenovirus, adeno-associated virus, herpes virus, vaccinia virus and retrovirus. Also preferred is liposomally-encapsulated **expression vector**.

DETD . . . variety of direct, local and regional approaches may be taken. For example, an organ may be directly injected with the **expression vector**. Also, a tumor bed may be treated prior to, during or after resection. Following resection, one generally will deliver the. . .

DETD Where clinical applications are contemplated, it will be necessary to prepare pharmaceutical compositions--**expression vectors**, virus stocks, proteins, antibodies and drugs--in a form appropriate for the intended application. Generally, this will entail preparing compositions that. . .

DETD Graham and Prevec, "Adenovirus-based **expression vectors** and recombinant vaccines," Biotechnology, 20:363-390, 1992

DETD Ridgeway, "Mammalian **expression vectors**," In: Rodriguez R L, Denhardt D T, ed. Vectors: A survey of molecular cloning vectors and their uses. Stoneham: Butterworth,. . .

=> d bib abs l22 2

L22 ANSWER 2 OF 6 USPATFULL
AN 1999:43465 USPATFULL
TI Methods and compositions for inhibiting hexokinase
IN Newgard, Christopher B., Dallas, TX, United States
Han, He-Ping, Dallas, TX, United States
Becker, Thomas C., Carrollton, TX, United States
Wilson, John E., East Lansing, MI, United States
PA Betagene, Inc., Dallas, TX, United States (U.S. corporation)
Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5891717 19990406
AI US 1996-588976 19960119 (8)
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Moore, William W.
LREP Arnold, White & Durkee
CLMN Number of Claims: 79
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 5470
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are compositions and methods for inhibiting hexokinase enzymes in mammalian cells. Specifically provided are proteins that stimulate the production of trehalose-6-phosphate and their respective genes; hexokinase-specific ribozymes and genes encoding such constructs; and agents that competitively reduce hexokinase activity, e.g., by displacing hexokinase from mitochondria, and their respective genes. The latter group of agents includes inactive hexokinases and fragments thereof that retain mitochondrial binding functions and hexokinase-glucokinase chimeras that further substitute glucokinase activity for hexokinase activity. Mammalian cells including such hexokinase inhibitors, methods of making such cells and various in vitro and in vivo methods of using cells with reduced hexokinase activity are also described herein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d bib abs l22 3

L22 ANSWER 3 OF 6 USPATFULL
AN 1998:162337 USPATFULL
TI Hexokinase inhibitors
IN Newgard, Christopher B., Dallas, TX, United States
Han, He-Ping, Arlington, TX, United States
Normington, Karl D., Dallas, TX, United States
PA Board of Regents, The University of texas System, Austin, TX, United States (U.S. corporation)
Betagene, Inc., Dallas, TX, United States (U.S. corporation)
PI US 5854067 19981229
AI US 1996-588983 19960119 (8)
DT Utility
EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Wang, Andrew
LREP Arnold, White & Durkee
CLMN Number of Claims: 64
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 5377
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are compositions and methods for inhibiting hexokinase enzymes in mammalian cells. Specifically provided are proteins that stimulate the production of trehalose-6-phosphate and their respective genes; hexokinase-specific ribozymes and genes encoding such constructs; and agents that competitively reduce hexokinase activity, e.g., by displacing hexokinase from mitochondria, and their respective genes. The latter group of agents includes inactive hexokinases and fragments thereof that retain mitochondrial binding functions and hexokinase-glucokinase chimeras that further substitute glucokinase activity for hexokinase activity. Mammalian cells including such hexokinase inhibitors, methods of making such cells and various in vitro and in vivo methods of using cells with reduced hexokinase activity are also described herein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic l22 3

L22 ANSWER 3 OF 6 USPATFULL
SUMM . . . their mitochrondial binding sites within an intact cell. Such "displacing agents" will generally comprise a mitochondrial binding region from the **N-terminal** domain of a low K.sub.m hexokinase, such as hexokinase I or hexokinase II.
SUMM The term "mitochrondial binding region from the **N-terminal** domain," as used herein, includes constructs of between about 15 amino acids in length and about 455 amino acids in. . .
SUMM Constructs that consist essentially of the **N-terminal** domain of a low K.sub.m hexokinase will be preferred for use in certain aspects of the invention. This is based. . .
SUMM . . . themselves exhibit low K.sub.m hexokinase activity. These constructs will generally comprise a mitochondrial binding peptide, polypeptide or protein from the **N-terminal** domain of a low K.sub.m hexokinase operatively linked to at least the catalytic domain of a glucokinase enzyme (hexokinase IV).
DRWD FIG. 1. Western analysis of Hexokinase **N-terminal** half expression in RIN 1046-38 cells. Whole cell lysates were resolved by SDS-PAGE and immunoreactive proteins were detected using a. . . molecular weight of greater than 100 kD on this gel system. Lysates from five monoclonal RIN lines expressing the hexokinase **N-terminal** half are in lanes 3 through 7.
DETD . . . It is preferred that the engineered hexokinase or hexokinase-glucokinase chimera be provided to the cell by means of a eukaryotic **expression vector** that is introduced into the cell and that directs expression of the desired protein.
DETD . . . generating trehalose-6-phosphate, which is a metabolic

inhibitor of hexokinase activity. Engineering of the yeast gene encoding TPS1 into a eukaryotic **expression vector** and introduction of the vector into a mammalian cell is generally the preferred method of producing the trehalose-6-phosphate inhibitor.

DETD . . . acid with RNA degradative catalytic activity ("catalytic ribozyme"). Again, it is preferred to engineer the hexokinase-specific ribozyme into a eukaryotic **expression vector** and to introduce the vector into a mammalian cell, where it directs the destruction of hexokinase mRNA and reduces expression. . .

DETD . . . low K.sub.m hexokinases that are inhibited as disclosed herein. Engineering of a mammalian glucokinase cDNA or gene into a eucaryotic **expression vector** and introduction of the vector into a mammalian cell is generally the preferred method of producing the glucose-6-phosphate inhibitor.

DETD . . . K.sub.m enzymes. The two halves of the low K.sub.m hexokinases are commonly described as the C-terminal "catalytic" domain and the **N-terminal** "regulatory" domain. The C-terminal domain retains full catalytic activity when expressed independently of the **N-terminal** domain and also exhibits allosteric inhibition by glucose-6-phosphate. It is believed that the glucose-6-phosphate allosteric site of the C-terminal domain. . . the intact enzyme, and that allosteric regulation of the intact enzyme is conferred by the glucose-6-phosphate binding site of the **N-terminal** "regulatory" domain (Wilson, 1994).

DETD . . . loses its capacity for mitochondrial binding, and that enzyme treated in this manner is lacking in a portion of its **N-terminal** domain (Polakis and Wilson, 1985). The **N-terminal** sequences of both hexokinases I and II are relatively hydrophobic, and it has been shown that the hydrophobic N-terminus of. . .

DETD Subsequently, Gelb et al., (1992) demonstrated that a chimeric protein consisting of the **N-terminal** 15 amino acids of hexokinase I fused to chloramphenicol acetyltransferase was capable of binding to rat liver mitochondria, and that. . .

DETD While the results of Gelb et al. (1992) argue for the importance of this small **N-terminal** segment in targeting of hexokinase to mitochondria, others have suggested that other regions of the molecule may also be important. . . by Mg.sup.2+, an effect likely reflecting electrostatic interactions between the enzyme and the outer mitochondrial membrane (i.e., not involving the **N-terminal** 15 amino acids that are intercalated into the membrane). Therefore, the mitochondrial binding regions of HK have not been clearly. . .

DETD Constructs of the present invention may comprise the **N-terminal** 15 amino acids of a hexokinase enzyme, preferably hexokinase I or II, since this segment should be easily expressed in cells and retained as a stable peptide. Constructs comprising the entire **N-terminal** domain of either hexokinase I or hexokinase II, or the intact, full-length hexokinase I or II proteins that have been. . .

DETD The reason for preferring the **N-terminal** domain construct is that this element seems to comprise a complete structural domain, based upon studies in which this domain. . . glucose-6-phosphate (Wilson, 1994; Arora et al., 1993; White and Wilson, 1987; White and Wilson, 1990). This suggests that the intact **N-terminal** domain should fold and form a structure analogous to its structure in the full-length hexokinase I or II protein. As. . . the present inventors contemplate that this structure mediates attachment of the intact hexokinase protein to mitochondria, the intact, correctly folded **N-terminal** domain is a preferred embodiment of this invention.

DETD For embodiments involving the **N-terminal** domain, a segment comprising amino acids 1-455 is preferred because of a naturally occurring NcoI restriction enzyme site in the DNA sequence corresponding to amino acid 482. This NcoI site allows the fragment encoding the **N-terminal** domain to be easily isolated and subcloned, and also allows direct fusion of the **N-terminal** domain of hexokinase to the intact functional sequence of glucokinase via an NcoI site located at the AUG start codon. . .

DETD . . . location into which a selected gene is to be transferred.

Sequences homologous to the target gene are included in the **expression vector**, and the selected gene is inserted into the vector such that target gene homologous sequences are interrupted by the selected. . .

DETD Throughout this application, the term "**expression vector** or construct" is meant to include any type of genetic construct containing a nucleic acid coding for a gene product. . .

DETD . . . (TH2B) Histone; Mouse or Type I Collagen; Glucose-Regulated Proteins (GRP94 and GRP78); Rat Growth Hormone; Human Serum Amyloid A (SAA); **Troponin I** (TN I); Platelet-Derived Growth Factor; Duchenne Muscular Dystrophy; SV40 or CMV; Polyoma; Retroviruses; Papilloma Virus; Hepatitis B Virus; Human Immunodeficiency. . .

DETD One of the preferred methods for in vivo delivery involves the use of an adenovirus **expression vector**. "Adenovirus **expression vector**" is meant to include those constructs containing adenovirus sequences sufficient to support packaging of the construct and to express an. . .

DETD The **expression vector** comprises a genetically engineered form of adenovirus. Knowledge of the genetic organization or adenovirus, a 36 kB, linear, double-strained DNA. . .

DETD The **expression vectors** and delivery vehicles of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present. . .

DETD **Expression Vectors**

DETD The present example describes **expression vectors** that have been found to be particularly useful in the context of the invention.

DETD The **N-terminal** Domain of Hexokinase Inhibits Hexokinase Binding to Mitochondria

DETD . . . was isolated encoding the first 455 amino acids of Hexokinase I (SEQ ID NO:7). SEQ ID NO:7 represents the entire **N-terminal** half of the protein and should fold into a stable domain containing the hexokinase I non-catalytic, regulatory domain as well. . .

DETD 1. Clones Expressing the **N-terminal** Domain of Hexokinase I

DETD Stable G418 resistant clones of RIN 1046-38 transfected with pCB6/intron/HKNterm were screened for expression of the hexokinase **N-terminal** half by western analysis as described. A protein of 482 amino acids with a predicted molecular weight of 55 Kd. . .

DETD All five clones express the hexokinase **N-terminal** half protein at levels higher than endogenous hexokinase I. Overexpression is expected to be required to dislodge mitochondrial bound endogenous. . .

DETD 2. Effects of the **N-terminal** Domain of Hexokinase I

DETD The effects of overexpression of the hexokinase **N-terminal** half on endogenous hexokinase in RIN cells are analyzed using the hexokinase enzymatic assay procedure described in detail by Kuwajima. . .

DETD Unlike the chimeric hexokinase/glucokinase proteins described in Example III, the hexokinase **N-terminal** half is enzymatically inactive, but is competent to bind to mitochondria and dislodge endogenous hexokinase. This is expected to have. . .

DETD SEQ ID NO:8 is the resulting 2911 base sequence encoding a 919 amino acid fusion protein consisting of the **N terminal** 455 amino acids of Hexokinase I and the entire 465 amino acid sequence of liver glucokinase (SEQ ID NO:9). SEQ ID NO:11 is the resulting 2911 base sequence encoding a 919 amino acid fusion protein consisting of the **N terminal** 455 amino acids of Hexokinase I and the entire 465 amino acid sequence of islet glucokinase (SEQ ID NO:12).

DETD For transient transfection studies, cDNAs encoding chimeric hexokinase/glucokinase proteins consisting of the **N-terminal** domain of hexokinase I (amino acids 1-455) linked in frame to either the full length liver isoform of glucokinase (HK-liverGK,. . .

DETD Polakis and Wilson, "An intact **N-terminal** sequence is critical for binding rat brain hexokinase to mitochondria," Arch. Biochem. Biophys., 236:328-337, 1985.

=> d kwic l22 4

L22 ANSWER 4 OF 6 USPATFULL

SUMM The nucleic acid segments of the present invention may also comprise a recombinant vector or even a recombinant **expression vector** capable of replicating within a cell. In particular, the nucleic acid segment expressing a GRIM polypeptide on introduction into a. . . defined as comprising the nucleic acid sequence set forth in SEQ ID NO:1 or its complement, or as a recombinant **expression vector** capable of expressing a GRIM polypeptide on introduction into a host cell.

SUMM For use in mammalian cells, the control functions on the **expression vectors** are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently. . .

SUMM . . . also be integrated into the host genome, and in particular, the host cell may be defined as comprising a recombinant **expression vector** and expressing a GRIM polypeptide.

SUMM Of particular interest is the use of insect cells as a host for baculoviral **expression vectors**. Currently, the preferred baculovirus expression systems utilize the lytic insect virus known as Autographa californica multiply enveloped nuclear polyhedrosis virus.. . . and control sequences. This can be accomplished by replacing the baculoviral polyhedron gene with the cDNA to be expressed. Baculoviral **expression vectors** ordinarily include all the original baculoviral genes except the polyhedron gene and may include additional marker genes such as the. . .

SUMM . . . are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7, 293 and MDCK cell lines. **Expression vectors** for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to. . .

SUMM . . . certain broad aspects as a method of making a Drosophila GRIM polypeptide. This method comprises the steps of obtaining an **expression vector** containing a nucleic acid sequence encoding a GRIM polypeptide wherein the nucleic acid sequence is operatively linked to a promoter,. . .

DETD . . . rescue was dose dependent and similar to levels of rescue obtained by corresponding doses of genomic rpr DNA. Second, the **N-terminal** portion of GRIM shares conspicuous similarity to RPR, a protein already well established as an activator of cell death in. . .

DETD . . . function. The mechanism by which grim elicits the apoptosis program remains to be determined as does the functional significance of **N-terminal** motif shared between grim, rpr and hid. In contrast to reported alignments between RPR and some death domain proteins [Cleveland. . .

DETD . . . isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the **expression vector** 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have. . .

DETD . . . are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7, 293 and MDCK cell lines. **Expression vectors** for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to. . .

DETD . . . 1989
Glucose-Regulated Proteins
Chang et al., 1989
(GRP94 and GRP78)
Rat Growth Hormone
Larsen et al., 1986
Human Serum Amyloid A
Edbrooke et al., 1989
(SAA)
Troponin I (TN I)
Yutzey et al., 1989
Platelet-Derived Growth Factor
Pech et al., 1989

Duchenne Muscular Dystrophy
Klamut et al., 1990
SV40 Banerji et. . .
CLM What is claimed is:
12. The nucleic acid segment of claim 11, wherein said vector is a recombinant **expression vector** capable of expressing an apoptosis inducing polypeptide on introduction into a host cell.

19. A method of making a Drosophila apoptosis inducing polypeptide comprising the steps of: a) obtaining an **expression vector** containing a nucleic acid sequence encoding an apoptosis inducing polypeptide comprising an amino acid sequence of SEQ ID NO:2 or. . .

=> d bib abs l22 5

L22 ANSWER 5 OF 6 USPATFULL
AN 1998:108278 USPATFULL
TI High affinity mutants of nuclear factor-interleukin 6 and methods of use therefor
IN Brasier, Allan R., Galveston, TX, United States
PA Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5804445 19980908
AI US 1996-585197 19960111 (8)
DT Utility
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Nelson, Amy J.
LREP Arnold, White & Durkee
CLMN Number of Claims: 21
ECL Exemplary Claim: 19
DRWN 17 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 2246
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to inhibitors of the sequence specific transcription factor nuclear factor IL-6 (NF-IL6) and methods of use therefor. In particular, substitution mutants in the N-terminus of the NF-IL6 tryptic core domain are disclosed that have a higher binding affinity for the DNA binding site than does the wild-type sequence.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic l22 5

L22 ANSWER 5 OF 6 USPATFULL
SUMM . . . was the leucine zipper domain (residues 303-345) that constitutes the DNA binding domain of the polypeptide. Of this region, the **N-terminal** portion (266-272) was identified as being involved in complex stabilization.
DETD . . . for the aspartic acid residues of the CSSD. The coding sequence for the tryptic core domain is cloned into an **expression vector** and mutagenized using site-directed methodology.
DETD . . . for a gene product in which part or all of the nucleic acid sequence is capable of being transcribed. Typical **expression vectors** include bacterial plasmids or phage, such as any of the pUC or Bluescript.TM. plasmid series or, as discussed further below,. . .

DETD . . . Gene
.alpha.-Fetoprotein
.tau.-Globin
.beta.-Globin
c-fos
c-HA-ras
Insulin
Neural Cell Adhesion Molecule (NCAM)
.alpha.1-Antitrypsin
H2B (TH2B) Histone
Mouse or Type I Collagen
Glucose-Regulated Proteins (GRP94 and GRP78)
Rat Growth Hormone
Human Serum Amyloid A (SAA)
Troponin I (TN I)
Platelet-Derived Growth Factor
Duchenne Muscular Dystrophy
SV40
Polyoma
Retroviruses
Papilloma Virus
Hepatitis B Virus
Human Immunodeficiency Virus

Cytomegalovirus
Gibbon Ape Leukemia Virus

DETD In order to effect expression of nucleic acid constructs, the **expression vector** carrying the constructs must be delivered into a cell. As described above, the one mechanism for delivery is via viral infection where the **expression vector** is encapsidated in an infectious adenovirus particle. For non-infectious vectors, other means may be required.

DETD Several non-viral methods for the transfer of **expression vectors** into cultured mammalian cells also are contemplated by the present invention. These include calcium phosphate precipitation (Graham and Van Der. . .

DETD In one embodiment of the invention, the adenoviral **expression vector** may simply consist of naked recombinant vector. Transfer of the construct may be performed by any of the methods mentioned. . .

DETD Another embodiment of the invention for transferring a naked DNA **expression vector** into cells may involve particle bombardment. This method depends on the ability to accelerate DNA coated microprojectiles to a high. . .

DETD In a further embodiment of the invention, the **expression vector** may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous. . .

DETD . . . yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such **expression vectors** have been successfully employed in transfer and expression of a polynucleotide in vitro and in vivo, then they are applicable. . .

DETD Another mechanism for transferring **expression vectors** into cells is receptor-mediated delivery. This approach takes advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost. . .

DETD . . . and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that an adenoviral **expression vector** also may be specifically delivered into a cell type such as lung, epithelial or tumor cells, by any number of. . .

DETD . . . U.S. Pat. No. 5,399,346, and incorporated herein in its entirety, disclose ex vivo therapeutic methods. During ex vivo culture, the **expression vector** can express the antisense K-ras construct. Finally, the cells may be reintroduced into the original animal, or administered into a. . .

DETD Where clinical application of NF-IL6 inhibitors or **expression vectors** coding therefore is undertaken, it will be necessary to prepare the complex as a pharmaceutical composition appropriate for the intended. . .

DETD Aqueous compositions of the present invention comprise an effective amount of the inhibitory peptide or **expression vector** encoding the inhibitory peptide, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred. . .

DETD As mentioned above, a preferred form for delivery of an **expression vector** according to the present invention is via liposomes. Liposomes also may be used to deliver formulated peptides. "Liposome" is a. . .

DETD The inhibitory peptides and **expression vectors** of the present invention may include classic pharmaceutical preparations. Administration of therapeutic compositions according to the present invention will be. . .

DETD (b) Construction of Alanine substituted NF-IL6 CSSD **expression vectors**

DETD The **expression vector** for the NF-IL6 tryptic core domain peptides was constructed by ligating NcoI-BamHI restriction fragments containing the appropriate coding sequences into. . .

DETD Ridgeway, "Mammalian **expression vectors**," In: Vectors: A survey of molecular cloning vectors and their uses, Rodriguez & Denhardt (eds.), Stoneham: Butterworth, pp. 467-92, 1988.

=> d kwic l22 6

L22 ANSWER 6 OF 6 USPATFULL

SUMM . . . family bind an MEF-1 sequence motif found in many skeletal muscle specific genes, for example creatine kinase and skeletal fast **troponin I**.

DETD . . . Lys.sup.108, shown boxed in FIG. 3. Two regions of divergence between RTEF-1 and human NTEF-1 are seen in the acidic **N-terminal** domain (Thr.sup.9 to Asn.sup.36) and in the proline-rich domain (Pro.sup.150 to Pro.sup.210) carboxy-terminal to the TEA domain. Despite the overall. . . these domains are retained in chicken RTEF-1A compared to human NTEF-1: 6 versus 7 acidic residues are conserved in the **N-terminal** and 13 versus 16 proline residues are conserved in the proline rich domain. These regions are two components of at. . .

DETD . . . hand, NTEF-1 activation function could be demonstrated in HeLa cells using vectors expressing GAL4/NTEF-1 chimeras transfected at low ratios of **expression vector** to GAL-4 dependent reporter.

DETD . . . activation of transcription in muscle and non-muscle cells, chimeras were constructed in which RTEF-1A and RTEF-1B, lacking the TEA and **N-terminal** domains, were fused to the DNA binding domain of GAL4. These fusion constructs were then cotransfected into cultured embryonic skeletal. . .

DETD . . . for example, in Sambrook et al., Molecular Cloning, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. Conveniently available **expression vectors** which include the replication system and transcriptional and translational regulatory sequences together with an insertion site for the TEF-1 DNA sequence may be employed. Examples of workable combinations of cell lines and **expression vectors** are described in Sambrook et al. In some circumstances, an inducible promoter may be preferred.

DETD Chimeric (fusion) **expression vectors** were constructed in the plasmid pGAL4mpolyII, Webster, H. J. G, et al., Cell (1988) 54: 199-207. The entire carboxy terminus. . . RTEF-1A or RTEF-1B isoforms from Val.sup.101 was fused to the DNA binding domain of GAL4 (amino acids 1-147). These chimeric **expression vectors** were cotransfected with 2 .mu.g of a GAL4-dependent CAT reporter, (17 mer.times.2) .beta.globinCAT (Webster, H. J. G., supra) into cultured. . .

=> d bib abs 127

L27 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
AN 1999:405082 CAPLUS
DN 131:54754
TI Single-chain polypeptide comprising **troponin** I and **troponin** C, and its use in **troponin** assays
IN **Shi, Qinwei**; Song, Qian-Li
PA Spectral Diagnostics, Inc., Can.
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9931235	A1	19990624	WO 1998-IB2095	19981218
	W: AU, CA, JP, MX				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9914446	A1	19990705	AU 1999-14446	19981218
PRAI	US 1997-993380		19971218		
	WO 1998-IB2095		19981218		

AB This invention provides a fusion protein comprising human cardiac **troponin** I and **troponin** C on the same polypeptide chain, thereby conferring conformational stability and immunostability to the product. The polypeptide preferably includes a linker sequence of about 6 to about 30 amino acids interposed between the sequences of **troponin** I and **troponin** C, chosen so that it does not interfere with the tertiary structure of the product and therefor its aforementioned utilities. Thus, the polypeptide of this invention provides a stable, reproducible, and easily purified material for the development of **troponin** assays, as well as material for use as controls and calibrators for said assays, and antigen for prepg. **troponin** antibodies.

=> d bib abs l27 2

L27 ANSWER 2 OF 20 USPATFULL
AN 1999:141656 USPATFULL
TI Single-chain polypeptides comprising creatine kinase M and creatine kinase B
IN **Shi, Qinwei**, Etobicoke, Canada
Tobias, Rowel, Mississauga, Canada
PA Spectral Diagnostics, Inc., Toronto, Canada (non-U.S. corporation)
PI US 5981249 19991109
AI US 1998-18760 19980205 (9)
DT Utility
EXNAM Primary Examiner: Achutamurthy, Ponnathapura; Assistant Examiner: Monshipouri, Maryam
LREP Klauber & Jackson
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 603
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to single-chain polypeptides and their genetic sequences comprising creatine kinase M and creatine kinase B. The single-chain polypeptide may be expressed recombinantly. A linker peptide may be interposed between the creatine kinase sequences. A linker peptide of about 6 to about 50 amino acids is preferred. The single-chain polypeptide has utility as a control or calibrator for creatine kinase MB assays, for the purification of creatine kinase antibodies, and as an antigen for the preparation of antibodies.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l27 2 kwic

L27 ANSWER 2 OF 20 USPATFULL
IN **Shi, Qinwei**, Etobicoke, Canada
DETD . . . example, as described by Hu et al. (1996, Protein Expression and Purification 7:289-293) in which rare codons in human cardiac **troponin** T were replaced with synonymous major codons. These methods are well known to the skilled artisan.

=> d bib abs l27 3

L27 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2000 ACS
AN 1999:725727 CAPLUS
TI Extra leader sequence affects immunoactivity of cardiac **troponin** I
AU **Liu**, **S**.; Zhang, M. Y.; Song, Q.; Zhang, X.; Kadijevic, L.; **Shi**, **Q**.
SO Clin. Chem. (Washington, D. C.) (1999), 45(11), 2045
CODEN: CLCHAU; ISSN: 0009-9147
PB American Association for Clinical Chemistry
DT Journal; Errata
LA English
AB Unavailable

=> d bib abs l27 4

L27 ANSWER 4 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
AN 1999388194 EMBASE
TI Erratum: Extra leader sequence affects immunoactivity of cardiac **troponin** I (Clinical Chemistry (1999) 45: (1300-1302)).
AU **Liu S.**; Zhang M.Y.; Song Q.; Zhang X.; Kadijevic K.; **Shi Q.**
SO Clinical Chemistry, (1999) 45/11 (2045).
ISSN: 0009-9147 CODEN: CLCHAU
CY United States
DT Journal; Errata
FS 018 Cardiovascular Diseases and Cardiovascular Surgery
LA English

=> d bib abs l27 5

L27 ANSWER 5 OF 20 MEDLINE DUPLICATE 2
AN 1999359316 MEDLINE
DN 99359316
TI Extra leader sequence affects immunoactivity of cardiac **troponin** I.
AU **Liu S**; Zhang M Y; Song Q; Zhang X; Kadijevic L; **Shi Q**
CS Spectral Diagnostics, Inc., 135-2 The West Mall, Toronto ON M9C 1C2, Canada.. sliu@spectraldiagnostics.com
SO CLINICAL CHEMISTRY, (1999 Aug) 45 (8 Pt 1) 1300-2.
Journal code: DBZ. ISSN: 0009-9147.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199910
EW 19991003

=> d bib abs l27 6

L27 ANSWER 6 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:395215 BIOSIS
DN PREV199900395215
TI An evaluation of cardiac **troponin** I and myoglobin/carbonic anhydrase III as markers of myocardial injury.
AU Tsang, M. (1); McClure, S. (1); Morin, P. (1); Shaikh, N. (1); **Liu, S. G. (1)**; Ash, J. (1); Kadijevic, L. (1); Styba, G. (1)
CS (1) Spectral Diagnostics Inc., Toronto, ON Canada
SO Clinical Chemistry, (June, 1999) Vol. 45, No. 6 PART 2, pp. A146. Meeting Info.: 51st Annual Meeting of the American Association of Clinical Chemistry New Orleans, Louisiana, USA July 25-29, 1999 American Association of Clinical Chemistry . ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs l27 7

L27 ANSWER 7 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:398363 BIOSIS
DN PREV199900398363
TI Cardiac **troponin** T expression in renal tissue.
AU **Ling**, M. M. (1); **Shi**, Q. W. (1); Yang, T. A. (1); Keffer, J. H. (1)
CS (1) Spectral Diagnostics Inc., Toronto, ON Canada
SO Clinical Chemistry, (June, 1999) Vol. 45, No. 6 PART 2, pp. A140. Meeting Info.: 51st Annual Meeting of the American Association of Clinical Chemistry New Orleans, Louisiana, USA July 25-29, 1999 American Association of Clinical Chemistry . ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs 127 8

L27 ANSWER 8 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:376964 BIOSIS
DN PREV199900376964
TI Recombinant single chain cardiac **troponin** I-C polypeptide: An ideal stable control material for cardiac **troponin** I immunoassays.
AU Zhang, M. Y. (1); Song, Q. L. (1); **Shi, Q. W. (1)**; Kadijevic, L. (1); **Liu, S. G. (1)**
CS (1) Spectral Diagnostics, Inc., Toronto, ON Canada
SO Clinical Chemistry, (June, 1999) Vol. 45, No. 6 PART 2, pp. A53-A54. Meeting Info.: 51st Annual Meeting of the American Association of Clinical Chemistry New Orleans, Louisiana, USA July 25-29, 1999 American Association of Clinical Chemistry
. ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs l27 9

L27 ANSWER 9 OF 20 USPATFULL DUPLICATE 3
AN 1998:138655 USPATFULL
TI Stable **troponin** subunits and complexes
IN **Liu, Shigui**, Toronto, Canada
Shi, Qinwei, Etobicoke, Canada
PA Spectral Diagnostics, Inc., Toronto, Canada (non-U.S. corporation)
PI US 5834210 19981110
AI US 1997-961858 19971031 (8)
RLI Continuation-in-part of Ser. No. US 1997-862613, filed on 23 May 1997, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Mayhew, Bradley S.
LREP Klauber & Jackson
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 809
AB Stable **troponin** subunits and complexes and methods for their preparation are described. Among other uses, these subunits and complexes are useful as antigens for the preparation of antibodies, and as controls and calibrators for **troponin** assays. One complex comprises a modified human cardiac **troponin** I together with human cardiac **troponin** T and human cardiac **troponin** C. Another complex comprises a modified human cardiac **troponin** I with human cardiac **troponin** C.

=> d bib abs l27 10

L27 ANSWER 10 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:339917 BIOSIS
DN PREV199800339917
TI The rapid quantitative determination of **troponin** I in whole blood using a fluorescence capillary fill immunosensor.
AU Laurino, J. P. (1); Ash, J. (1); Styba, G. (1); **Shi, Q. (1)**; Usategui, M. (1); Fletcher, J.; Milner, A.; Bacarese-Hamilton, T.
CS (1) Spectral Diagnostics Inc., Toronto, ON Canada
SO Clinical Chemistry, (June, 1998) Vol. 44, No. 6 PART 2, pp. A121. Meeting Info.: 50th Annual Meeting of the American Association of Clinical Chemistry Chicago, Illinois, USA August 2-6, 1998
ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs l27 11

L27 ANSWER 11 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:339473 BIOSIS
DN PREV199800339473
TI Development and analysis of recombinant human cardiac **troponin** complexes for immunoassay controls and calibrators.
AU **Liu, S. G.**; **Shi, Q. W.**; Song, Q. L.; Zhang, M. Y.; Zhang, X. C.; Kadijevic, L.; Laurino, J.; Keffer, J.
SO Clinical Chemistry, (June, 1998) Vol. 44, No. 6 PART 2, pp. A21. Meeting Info.: 50th Annual Meeting of the American Association of Clinical Chemistry Chicago, Illinois, USA August 2-6, 1998 ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs l27 12

L27 ANSWER 12 OF 20 MEDLINE DUPLICATE 4
AN 97462500 MEDLINE
DN 97462500
TI Analytical performance and clinical utility of a sensitive immunoassay for determination of human cardiac **troponin** I.
AU Davies E; Gawad Y; Takahashi M; **Shi Q**; Lam P; Styba G; Lau A; Heeschen C; Usategui M; Jackowski G
CS Spectral Diagnostics Inc., Toronto, Ontario,.Canada.
SO CLINICAL BIOCHEMISTRY, (1997 Aug) 30 (6) 479-90.
Journal code: DBV. ISSN: 0009-9120.
CY United States
DT (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
EW 19980104
AB OBJECTIVES: To determine the serum and plasma level of human cardiac **troponin** I (cTnI) resulting from myocardial damage, we have developed a sensitive and specific one-step enzyme immunoassay to measure cardiac **troponin** I. DESIGN AND METHODS: The COBAS cTnI assay is a semi-automated one-step solid phase immunoassay compatible with the COBAS Core. The assay is performed in a sandwich type format using a polyclonal goat antibody capture and two highly specific horseradish peroxidase conjugated monoclonal antibody detectors directed against different epitopes of the cTnI molecule. Calibrators were made with purified recombinant cTnI. RESULTS: The level of cTnI was determined in 84 healthy donors with no evidence of myocardial injury, resulting in a lower limit of detection (LLD) of 0.09 microgram/L. The upper reference limit (URL) of the normal reference range was calculated as 0.20 microgram/L. The dynamic range of the consequent EIA was between 0.09 and 6.0 micrograms/L with a total assay time of 45 min. Intra-assay and inter-assay variances (CVs) were < or = 4%. Cross-reactivity with fast and slow skeletal **troponin** I was absent in concentrations up to 2.0 mg/L. Common interferents yielded negative results in the cTnI assay. Clinical utility was confirmed by measuring the circulating serum or plasma levels of cardiac **troponin** I in serial samples from marathon runners, clinical samples from trauma patients, and patients presenting to the Emergency Department with complaints of chest pain. Results were further evaluated using clinical diagnosis at discharge and quantified concentrations of other cardiac markers by a Stratus analyzer and ELISA procedures. CONCLUSIONS: Results from normal and clinical samples assayed in house for cTnI concentrations indicate that the Spectral EIA is a highly sensitive means of quantifying cTnI levels in serum and plasma for acute cardiac syndrome. The cardiac specificity of cTnI over other well-known cardiac markers is reflected in experimental results and parallel clinical diagnosis.

=> d bib abs l27 13

L27 ANSWER 13 OF 20 MEDLINE DUPLICATE 5
AN 97462496 MEDLINE
DN 97462496
TI Removal of endotoxin from recombinant protein preparations.
AU **Liu S**; Tobias R; McClure S; Styba G; **Shi Q**; Jackowski G
CS Spectral Diagnostics, Inc., Toronto, ON, Canada.
SO CLINICAL BIOCHEMISTRY, (1997 Aug) 30 (6) 455-63.
Journal code: DBV. ISSN: 0009-9120.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
EW 19980104
AB OBJECTIVES: To develop an effective method to remove endotoxin from large scale E. coli recombinant protein purifications. DESIGN AND METHODS: Triton X-114 phase separation, affinity chromatography utilizing immobilized polymyxin B or immobilized histidine, were used to remove endotoxin from purified preparations of recombinant CK-BB, CK-MB, CK-MM, myoglobin, and cardiac **troponin** I. Endotoxin levels were measured by a Limulus Amebocyte Lysate gel-clot assay. The immunoactivity of these protein preparations was determined by BIAcore analysis using a panel of in-house generated monoclonal antibodies and by a Stratus Fluorometric Analyzer. In the case of **troponin** I, the BIAcore was also utilized to measure **troponin** C interactions. RESULTS: Phase separation with Triton X-114 was the most effective method in reducing the amount of endotoxin present in the protein preparations compared to either polymyxin B or histidine affinity chromatography. With Triton X-114, the reduction in endotoxin levels was greater than 99% and recovery of the proteins after endotoxin removal was greater than 90%. All three procedures for removing endotoxin had no deleterious effects on the immunoactivity of majority proteins when tested with a panel of monoclonal antibodies. **Troponin** I also retained its ability to bind to **troponin** C in the presence of Ca2+. Recombinant CK-BB and CK-MM which were expressed in the soluble fraction of E. coli cell lysates, contained significantly higher endotoxin levels than recombinant CK-MB, myoglobin and cardiac **troponin** I which were expressed in the form of inclusion bodies. CONCLUSION: Of the three methods tested, Triton X-114 phase separation was the most effective way of removing endotoxin from recombinant proteins.

=> d bib abs l27 14

L27 ANSWER 14 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:334521 BIOSIS
DN PREV199799633724
TI Over-expression, purification and refolding of recombinant human fast skeletal **troponin** I.
AU **Liu, S.**; **Shi, Q.**; Styba, G.; Jackowski, G.
CS Res. Development, Spectral Diagnostics Inc., Toronto, ON Canada
SO Clinical Chemistry, (1997) Vol. 43, No. 6 PART 2, pp. S158.
Meeting Info.: 49th Annual Meeting of the American Association for Clinical Chemistry Atlanta, Georgia, USA July 20-24, 1997
ISSN: 0009-9147.
DT Conference; Abstract; Conference
LA English

=> d bib abs l27 15

L27 ANSWER 15 OF 20 MEDLINE DUPLICATE 6
AN 96426681 MEDLINE
DN 96426681
TI Use of enzyme immunoassay for measurement of skeletal **troponin**-I utilizing isoform-specific monoclonal antibodies.
AU Takahashi M; Lee L; **Shi Q**; Gawad Y; Jackowski G
CS Spectral Diagnostics, Inc., Toronto, Ontario, Canada.
SO CLINICAL BIOCHEMISTRY, (1996 Aug) 29 (4) 301-8.
Journal code: DBV. ISSN: 0009-9120.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199704
EW 19970403
AB OBJECTIVE: To determine the serum level of fast skeletal **troponin** I (fsTnl) resulting from skeletal muscle damage, we have developed a sensitive two-site enzyme immunoassay to measure skeletal **troponin** I. DESIGN AND METHODS: Twelve monoclonal antibodies were raised against human fsTnl. Of these antibodies, 8 were fsTnl-specific and the remaining 4 reacted with both skeletal and cardiac **troponin** I (cTnl). Two monoclonals were utilized for a development of this fsTnl immunoassay. Standards were made with purified recombinant human fsTnl for the range of 0-25 micrograms/mL. RESULTS: Total assay variance (CV) ranged from 1.7% to 9.6%. The upper limit of the normal reference range was established as 0.2 microgram/L by determining fsTnl concentration in sera of 108 healthy donors without evidence of muscle damage. Purified human cTnl up to 500 micrograms/L and cTnl-positive clinical serum samples yielded negative results in the fsTnl assay. The serum levels of fsTnl were determined in trauma patients, patients with chronic degenerative muscle disease, and marathon runners. In the study populations, the serum levels of fsTnl were correlated with other biochemical markers that are traditionally used to monitor striated muscle damage. CONCLUSIONS: In the present preliminary studies, measuring the serum levels of fsTnl in patients with various forms of muscle damage is more accurate than using the classical non muscle-specific biochemical markers.

=> d bib abs l27 16

L27 ANSWER 16 OF 20 MEDLINE DUPLICATE 7
AN 97013819 MEDLINE
DN 97013819
TI Specific replacement of consecutive AGG codons results in high-level expression of human cardiac **troponin** T in Escherichia coli.
AU Hu X; **Shi Q**; Yang T; Jackowski G
CS Spectral Diagnostics Inc., Toronto, Ontario, Canada.
SO PROTEIN EXPRESSION AND PURIFICATION, (1996 May) 7 (3) 289-93.
Journal code: BJV. ISSN: 1046-5928.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199703
AB The adult isoform of human cardiac **troponin** T (TnT) contains 288 amino acids, 14 of which (4.9%) are encoded by the rarely used arginine codons (12 AGG, 2 AGA) in Escherichia coli genes. To generate sufficient quantity of TnT protein for antibody production, we cloned the corresponding cDNA and expressed it in E. coli. A low-level expression of TnT that comprised only about 1% of total cell protein was initially observed with the use of the native cDNA. The existence of two pairs of consecutive AGG codons AGG(165) AGG(166) and AGG(215) AGG(216) in the cDNA was suspected to be the main cause for this low-level expression. These two pairs of consecutive AGG codons were successively replaced with the major synonymous codon CGT by site-directed mutagenesis. As suspected, a 10-fold increase in TnT expression was obtained when one pair of the rare arginine codons was replaced and a 40-fold increase was achieved when both pairs of the rare codons were replaced. Our finding demonstrates the importance of consecutive rare codons in the suppression of high-level expression of heterologous proteins in E. coli and suggests that in order to maximize protein expression, a similar approach may be taken with other genes which contain consecutive rare codons.

=> d bib abs 127 17

L27 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2000 ACS
AN 1997:599048 CAPLUS
DN 127:216095
TI Correlation between changes of plasma content of ET-1 and myocardial **troponin** T in patients with CO poisoning
AU Pang, Jien; Fan, Hengliang; Yu, Hongwei; Gao, Guangkai; Qu, Ning; He, Tao; **Liu, Song**
CS PLA NO. 401 Hospital, QingDao, 266071, Peop. Rep. China
SO Zhongguo Gonggong Weisheng Xuebao (1996), 15(5), 276-277
CODEN: ZGWXEQ; ISSN: 1001-0572
PB Zhongguo Gonggong Weisheng Zazhi Chubanshe
DT Journal
LA Chinese
AB In this study, the plasma contents of endothelin-1 (ET-1) and the serum contents of cardiac specific **troponin** T (cTnT) were measured in a series of 26 acute CO poisoning patients. A control group of 30 healthy volunteers was set too. The results showed that significant difference (P<0.01) of plasma ET-1 was present in the patients with varied Glasgow scores. Significant difference (P<0.001) of cTnT exists between the percentage of apparent myocardial cell damage (limit value:cTnT.gtoreq.0.2.mu.g/L) in severe, moderate and mild cases. There is good pos. correlation(P<0.05) between ET-1 and cTnT. These results reveal: (1) The content of plasma ET-1 can be used as an index for the assessment of disease severity in acute CO poisoning patients, so can be the content of serum cTnT. (2) The high level of plasma ET-1 may play an important role in myocardial cell damage in CO poisoning patients.

=> d bib abs l27 18

L27 ANSWER 18 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1995:333695 BIOSIS
DN PREV199598347995
TI Purification and biacore analysis of recombinant (rTNI) and native (nTNI) cardiac **troponin** I.
AU Styba, Garth; Yang, Jianying; **Shi, Qinwei; Liu, Shigui Jeremy**; Jackowski, George
CS Spectral Diagn., Toronto M9C 1C2 Canada
SO Clinical Chemistry, (1995) Vol. 41, No. S6 PART 2, pp. S152.
Meeting Info.: 47th Annual Meeting of the American Association for Clinical Chemistry, Inc. Anaheim, California, USA July 16-20, 1995
ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs 127 19

L27 ANSWER 19 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1995:333294 BIOSIS
DN PREV199598347594
TI Use of enzyme immunoassay for measurement of skeletal **troponin** I utilizing isoform-specific monoclonal antibodies.
AU Takahashi, M.; Lee, L.; **Shi**, **Qinwei**; Gawad, Y.; Jackowski, G.
CS Spectral Diagnostics Inc., Toronto, ON M9C 1C2 Canada
SO Clinical Chemistry, (1995) Vol. 41, No. S6 PART 2, pp. S61.
Meeting Info.: 47th Annual Meeting of the American Association for Clinical Chemistry, Inc. Anaheim, California, USA July 16-20, 1995
ISSN: 0009-9147.
DT Conference
LA English

=> d bib abs l27 20

L27 ANSWER 20 OF 20 MEDLINE
AN 91291758 MEDLINE
DN 91291758
TI An embryonic origin for medulloblastoma.
AU Valtz N L; Hayes T E; Norregaard T; **Liu S M**; McKay R D
CS Department of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge 02139.
SO NEW BIOLOGIST, (1991 Apr) 3 (4) 364-71.
Journal code: AZH. ISSN: 1043-4674.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199110
AB Medulloblastoma is a common brain tumor of children. Three differentiated cell types are found in medulloblastomas: neurons, glia, and muscle cells. Because of the presence of multiple differentiated cell types these tumors were named after a postulated cerebellar stem cell, the medulloblast, that would give rise to the differentiated cells found in the tumors. We describe a cell line with the properties expected of the postulated medulloblast. The rat cerebellar cell line ST15A expresses an intermediate filament, nestin, that is characteristic of neuroepithelial stem cells. ST15A cells can differentiate, gaining either neuronal or glial properties. In this paper we show that the same clonal cell can also differentiate into muscle cells. This result suggests that a single neuroectodermal cell can give rise to the different cell types found in medulloblastoma. We also show expression of nestin in human medulloblastoma tissue and in a medulloblastoma-derived cell line. Both the properties of the ST15A cell line and the expression of nestin in medulloblastoma support a neuroectodermal stem cell origin for this childhood tumor.